a size range of droplets <20-μm. Solutions of different densities were prepared from ethanol (ρ=0.78 g/ml) or concentrated $CaCl_2$ solutions (ρ=1.12 and 1.23 g/ml); viscosities of these solutions varied from 1 to 3 centipoise.

[0260] Cells (H1650 lung cancer cell line) were cultured in RPMI 1640 media with 10% FBS and trypsinized and resuspended in PBS prior to use. Whole blood was collected from a healthy volunteer in EDTA coated vacutainer tubes by a trained phlebotomist. Blood was diluted in PBS to 1-5% for experiments. Cells were dyed using either calcein AM (5 μM), a cytoplasmic dye, or Hoescht 33342 (1 μM), which is a cell permeable DNA dye.

[0261] Microfabrication

[0262] Exemplary devices described herein were fabricated using standard soft lithography techniques. Briefly, SU-8 2035 was spun at 2250 rpm for 30 seconds to create a 50 μm thick layer on a 10 cm silicon wafer. Thickness was measured using a microscope with a metered focus and varied between 42-56 μm across a wafer. The pattern was photolithographically defined in this layer using a mylar mask printed at 40,000 dpi (See Supplementary AutoCAD files). After development PDMS was poured onto the SU-8 master at a 10 to 1 ratio of base to crosslinker, degassed in a vacuum chamber, and cured at 65 degree C. overnight. The devices were then cut from the mold; ports were punched with a sharpened flat tip needle, and then bonded to glass slides or cover glass using oxygen plasma. After plasma treatment and placement onto the glass substrate the devices were maintained at 70 degree C. on a hotplate for 15 minutes to increase bonding.

[0263] Dimensionless Numbers

[0264] For a straight rectangular channel the Re, a ratio between the inertial and viscous forces can be easily defined as $\rho U D_h / \mu$ where ρ is the density of the fluid, U is the mean velocity, and D_h, the hydraulic diameter, is defined as 2ab/(a+b). With a and b being the width and height of the channel. However, for curving channels and asymmetric curving channels taking only a rectangular cross-section and considering the R_e for this will overestimate the inertial effects. In order to define a correct R_e for these geometries fluid dynamic simulations were conducted of the geometry using COMSOL Multiphysics. A R_e was determined from the balance of inertial to viscous forces for node points within the middle of the stream. This method yielded the analytical R_e for straight rectangular channels as well. In the case of the asymmetric channels the R_e differs in the small or large curving turn and for simplicity a single R_e was used corresponding to the small turn throughout this work. As an example an average velocity of 42 cm/sec corresponds to a R_e of 5 in a 50 μm×50 μm small curving channel of radius of curvature, r=40 μm, while R_e=20 for a straight rectangular channel. Dean numbers were also calculated using these simulated R_e.

[0265] Particle Localization

[0266] The bias and accuracy of localization based on fitting to a functional form will depend on the pixel size (i.e. the level of sampling) and the signal to noise of the system (S/N). S/N is defined as, $S/N=(I_o-I_b)/\sigma_o$, that is the average intensity of the background subtracted from the average intensity of the object and divided by the standard deviation or noise over the object. This is the highest noise region due to shot noise being proportional to the square root of the number of photoelectrons. For the system, with highly dyed fluorescent microspheres the S/N was determined to be 60 by taking the standard deviation of intensity levels of a single stream over distance. This is in contrast to systems imaging single molecules which have typical S/N of 4-10. For the signal to noise ratio and a pixel sampling size of 330 nm, a predicted accuracy of localization of ~3 nm is expected. This result allows confidence in localization measurements that are larger than this value by around an order of magnitude.

[0267] Image Analysis

[0268] For flow cytometry applications and to determine autocorrelation functions for flowing streams of particles Matlab (The Mathworks Inc.) was used to conduct image analysis of sequences of images. First, for each movie a kernel image was selected that was representative of an in focus particle. This kernel was then convolved with the image to form an intensity map with peaks at particle positions. A defined section of this intensity map that corresponded to the distance a particle traveled in a given frame was converted to a time series of intensity and appended onto an array with time series from previous frames. This process was repeated for each frame until a full time series was assembled of particle flow through the detection area. The temporal signal was used to determine an autocorrelation function to analyze the favored distances between particles and length of trains. It should be noted that convolution will necessarily increase the apparent width of a given particle, but was conducted to obtain single peaks at particle positions from the multiple intensity peak raw data.

[0269] Experimental Setup

[0270] As described herein, experiments to determine the distribution of particle positions within the channels were performed using time lapse fluorescence microscopy. Solutions were introduced into a syringe and connected by PEEK tubing to the PDMS devices. In one embodiment, the system included a filter region to remove any large debris, curving separation microchannels, and five collection outlets, as shown in FIG. **33**. In other embodiments, the system included multiple inlets and a single collection outlet. Outlets of PEEK tubing were also connected to the outlet ports of the device and routed into a waste container or collection tubes. Flow was driven by a syringe pump (Harvard Apparatus PHD 2000). In one embodiment, curving channels having a width of 350-μm on the small radius of curvature turn and a width of 650-μm on the large radius of curvature turn were used. The average radius of curvature on the narrow and wide turns is 325 and 890-μm, respectively. This geometry results in an asymmetric system with a Dean drag (F_D) that is ~8 times larger in the small radius turn than in the large turn. In the illustrated embodiment, the entirety of the separation channel is composed of 31 units consisting of one small and one large turn, wound into three straight segments (10-11 units each) to reduce the device footprint.

[0271] PDMS devices were mounted onto the stage of an inverted fluorescent microscope (Nikon TE2000-U). Fluorescent streak images were obtained with a cooled CCD camera (Spot RT, Diagnostic Instruments) using exposure times from 500-5000 ms, depending on particle concentration and flow rate. Images were collected in the Spot software and further analysis was conducted using ImageJ.

[0272] Confocal imaging was conducted in the same manner as inverted fluorescent imaging except devices were bonded to coverglass slides to allow objective access. A 40× objective was used with a pinhole diameter of 1.05 airy disks. The z-y plane was scanned 8 successive times with a residence time of 0.3 ms at each scan point to obtain the images.

[0273] High-speed camera imaging was conducted in the same manner as inverted fluorescent imaging except white